Efficient Selection of Unique and Popular Oligos for Large EST Databases

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joint work with

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General problem

- Input: A list of DNA sequences
- Output: A list of short DNA strings of length 20-50 bases (oligos)
  - occur only once in each DNA sequence ("unique" oligos problem)

  or

  - occur in as many DNA sequences as possible ("popular" oligos problem)

Barley genome (*H. vulgare*)

- Size is ~ $5x10^9$ bases
  - 12 times the size of Rice
  - 35 times the size of Arabidopsis
- Too large for whole sequencing
- Strategy
  - Build a BAC library of Barley
  - Identify/sequence only the BACs containing the genes (expected ~ 10%)

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Method

- An EST database for Barley is available
- Use the EST db to identify a set of "popular" oligos that hybridize with as many genes/EST as possible (maximize coverage)
- Use as little oligos/filter/screens as possible (minimize time and money)

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Objectives

- Maximize the *coverage ratio* (number of covered ESTs/number of oligos)
- Minimize the computational resources (memory, time)

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Barley EST db

- Composed by ~ 350K EST sequences
- Cleaned (quality-trimming, cleaned of contaminants, etc.)
- Assembled (pre-clustered, assembled)
- Final dataset (HarvEST v1.07)
  - 46,145 unigenes
  - 28,475,016 bases

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Related work

- Pattern discovery (Meme, Teiresias, Pratt, Gibbs, Projection, Weeder, etc.) cannot be used because of the large input size
- Primer/probe design typically use all-against-all BLAST (eg., [Li&Stormo'01], [Rouillard *et al.*'02]) are extremely slow
- Rahmann [CSB'02] uses suffix arrays (requires ~ 50 hours on Compaq Alpha with 16GB RAM on a dataset of 40Mbases)

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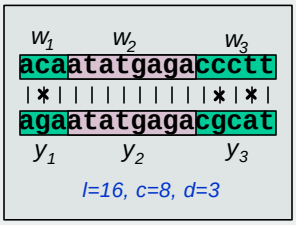
Def: *(c,d)*-match

- Given integers *c* and *d* and strings *w* and *y*, $|w|=|y|$, we say that *w* *(c,d)*-match *y* **iff** *w* and *y* can be partitioned in substrings $w=w_1w_2w_3$ and $y=y_1y_2y_3$ such that
- $|w_1|=|y_1|$ and $|w_3|=|y_3|$
- $w_2=y_2$, $|w_2|=|y_2|=c$ (core)
- $H(w_1w_3,y_1y_3)=d$



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Def: *(c,d)*-coverage

- Given a set $X=\{x_1,\ldots,x_k\}$, a string *y* and integers *c* and *d*, the *(c,d)-coverage* of *y* is the number of sequences of *X* containing each at least one *(c,d)*-match of *y*
- Integer *l* to denote the length of *y (l-mer)*

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"popular oligos" problem

- Given $X=\{x_1,\ldots,x_k\}$ and integers *l, d, c* and *T*, find all strings of length *l* such that their *(c,d)*-coverage in *X* is *=T*
- We call these strings "popular oligos"
- In our experiments
  l=36, c=20, d=2 or *3, T=2…50*

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Observations

- Note that a popular oligo may never appear exactly in *X*
- Enumerating/counting all $\binom{l-c}{d}(|\Sigma|-1)^d$ possible *(c,d)*-matches of each *l*-mer in *X* is computationally impractical
- For example, if *l=36, c=20*, *d=3,* $|\Sigma|=4$, one should count ~ 15K (20,3)-matches for each 36mer. We have 2*28M 36mers, for a total of 846B elementary operations

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Heuristics: phase one

- Build an hash table for the cores
- For each core w_2 that appears in $=T_c$ (core coverage threshold) sequences
  - Collect all flanking regions w_1w_3, such that $w_1w_2w_3$ is an *l*-mer with popular core w_2
  - Run phase two on set of all extensions w_1w_3

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Example: phase one

>EST0
TGGAGTCCTCGGACACGATCACATCGACAATGTGAA
GGCGA
>EST1
GTGAAGGAGGTAGATCAAATAGAGCCTGCCCTAAAA
AGGCAGCTTATAATCTCCACTGCT
>EST2
TCCGACTACTGCACCCCGAGCGGATCACACAATGGAA
GGCCCGTGCGC
>EST3
GTGAAGGAGGTAGATACTCGTATACGATCACTGCCTA
AAAAGGCAGCTTATAATCTCCATATCGCTG

l=8, *c*=5, *d*=1, T_c=3

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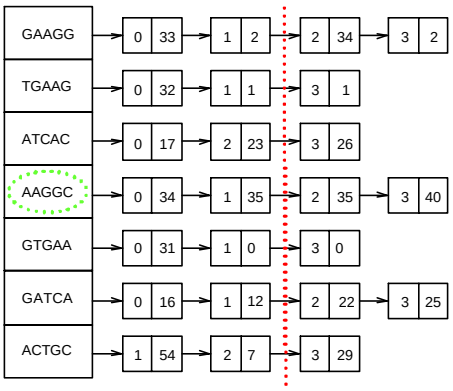
Example: phase one

>EST0
TGGAGTCCTCGGACACGATCACATCGACAATGTGAA
GGCGA
>EST1
GTGAAGGAGGTAGATCAAATAGAGCCTGCCCTAAAA
AGGCAGCTTATAATCTCCACTGCT
>EST2
TCCGACTACTGCACCCCGAGCGGATCACACAATGGAA
GGCCCGTGCGC
>EST3
GTGAAGGAGGTAGATACTCGTATACGATCACTGCCTA
AAAAGGCAGCTTATAATCTCCATATCGCTG



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Heuristics: phase two (UPGMA)

- Place all $w_1 w_3$ at the leaves of the tree & merge identical leaves
- Build the UPGMA* tree on Hamming distance
- Create a set of *d*-mutants for each string in the leaves of the tree
- Traverse the tree bottom-up performing set intersection
  - as soon as intersection is empty, separate the subtree from the rest of the tree
- The sets at the root of each tree in the forest represent the candidate popular oligo

* Unweighed Pair Group Method with Arithmetic Mean

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Example : phase two (UPGMA)

core **AAGGC**

occurrences

GTG **AAGGC** GA
AAA **AAGGC** AGC
TGG **AAGGC** CCG
AAA **AAGGC** AGC

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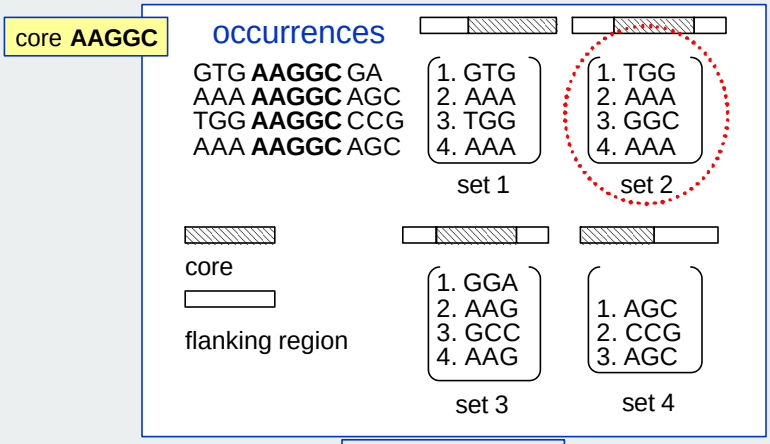
Example : phase two (UPGMA)



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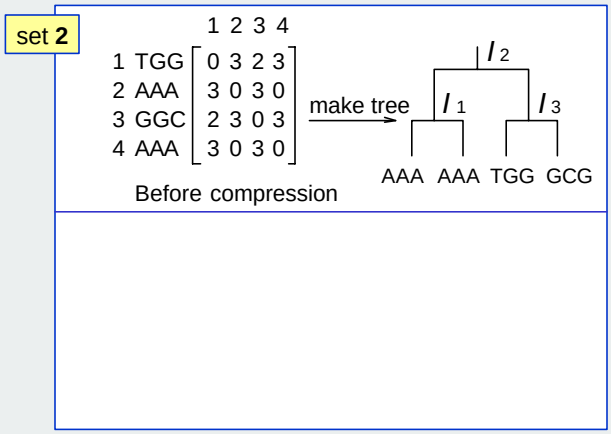
Example : phase two (UPGMA)



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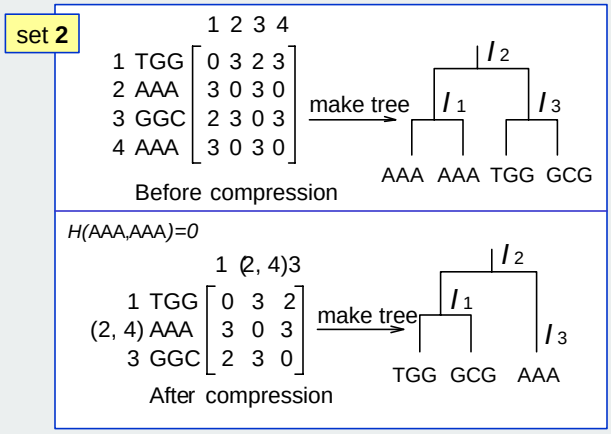

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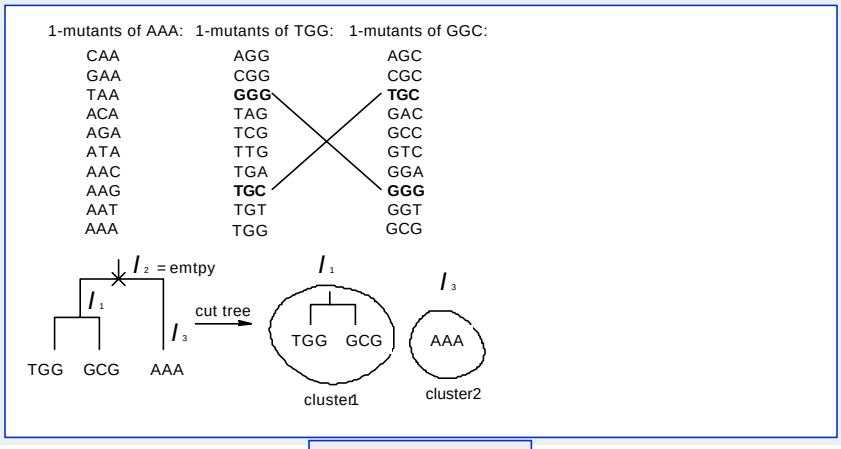
Example : phase two (UPGMA)



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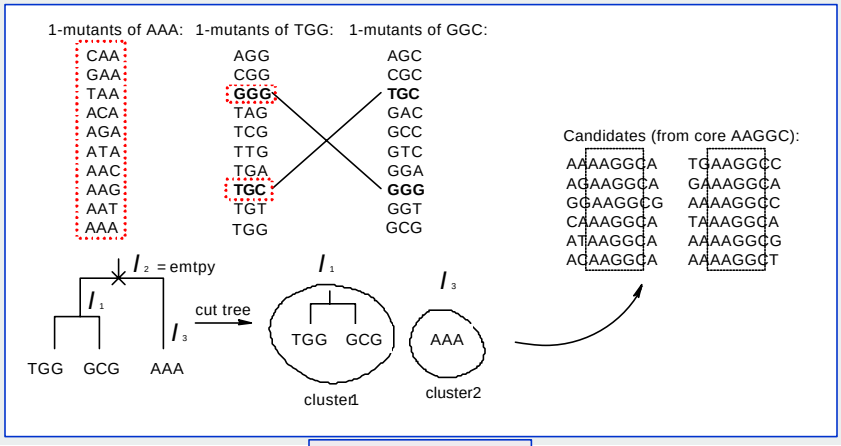
Example : phase two (UPGMA)



l=8, c=5, d=1, T_c=3

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Heuristics: phase three

- Radix sort the candidate oligos to remove duplicates
- Discard unsuitable oligos
  - low-complexity strings (polyA, polyT, etc.)
  - 44% < GC-content < 56%
- Compute coverage
- Compress/correct oligos

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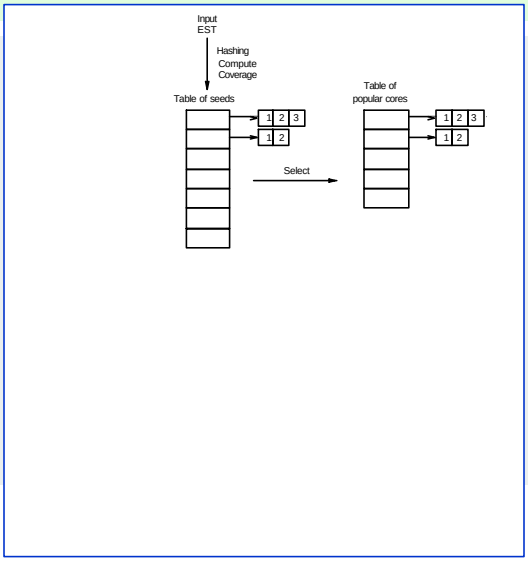
Overview: phase one



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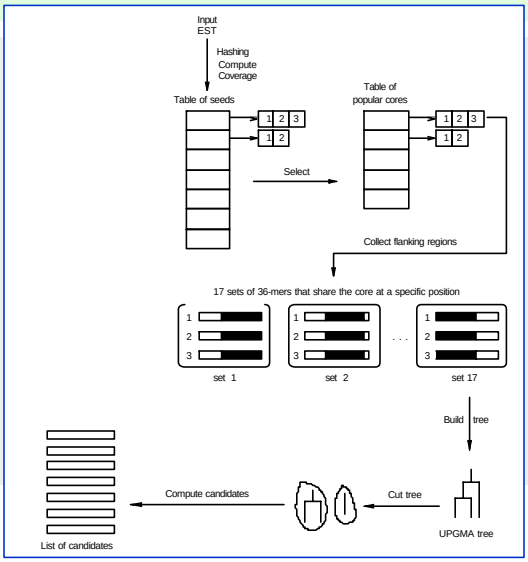
Overview: phase two (UPGMA)



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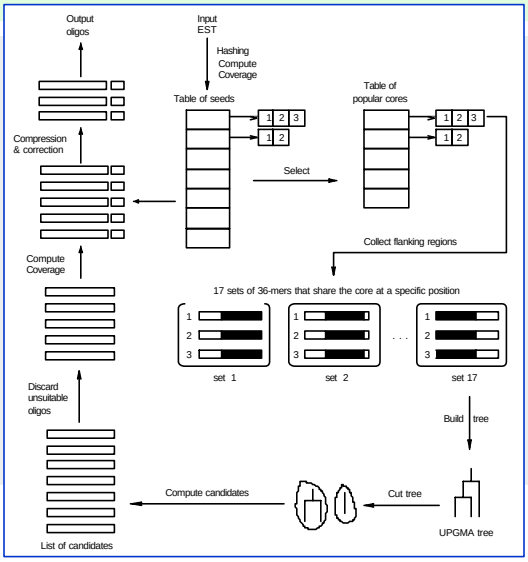
Overview: phase three



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Limitation of the heuristics

- Cores which have coverage below T_c are called unpopular
- A *l*-mer can *(c,d)*-match with any of its *l-c+1* cores
- We will miss popular oligos which popularity depend on a combination of several unpopular cores

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Simulations

- Generate $\{x_1,\ldots,x_k\}$ random sequences
- Inject $\{I_1,\ldots,I_s\}$ popular oligos with d errors outside a core of length c, with coverage $\{C_1,\ldots,C_s\}$ (Gaussian distribution, max coverage R)
- Run the popular oligo algorithm on $\{x_1,\ldots,x_k\}$

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Simulation

- Obtain $\{O_1,\ldots,O_t\}$ with coverage $\{C'_1,\ldots,C'_s\}$ (sorted)
- $\{O_1,\ldots,O_t\}$ is compressed
- Compare (I,C) with (O,C')
- For each $1=i=u$ for $u=min(s,t)$ we compute

$$E(C,C') = \frac{1}{u}\sum_{i=1}^{u}\frac{|C_i - C_i'|}{C_i'}$$

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Simulation results

- *k=2000, $|x_i|$=720, c=20, s=100, R=100*

*E*100*	*d=2*	*d=3*
T_c*=10*	1.89	5.30
T_c*=15*	0	0.07
T_c*=20*	0.6	0.06
T_c*=25*	0	0.31
T_c*=30*	0.07	0.25

- We never miss any oligo whose coverage is above T_c*+10*

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Experimental results

- *l=36* (oligo length)
- *c=20* (core length)
- *d=2,3* (max mismatches outside core)
- T_c*=varies* (core coverage threshold)
- *k=46,145* unigenes
- *n=28* million bases
- PC with 1.2 GHz CPU and 1GB memory

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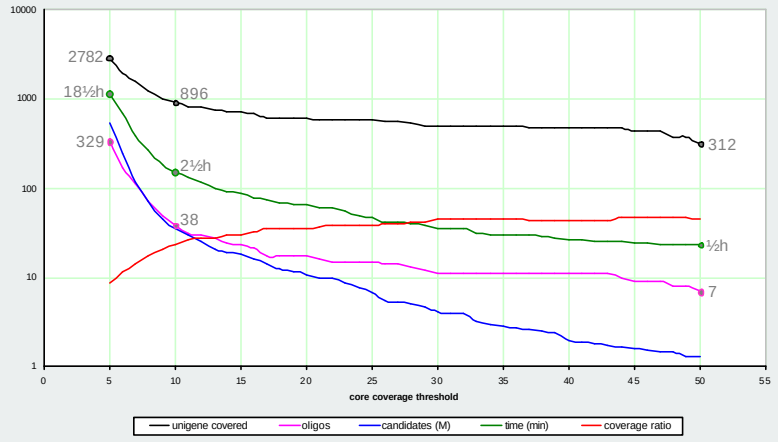
Coverage graph



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Current & Future work

- Progressive processing to reduce memory requirements
- Fine tuning & optimization of the code
- New strategies to improve coverage ratio
- New definition for popular/unique oligos
- Parallel implementation

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Complexity

- Build a seed table *O(cn)*
- Collect flanking substrings *O(nr(l-c))* where *r* is # occurrences of cores
- Building UPGMA $O\left(r\binom{l-c}{d}3^d\right)$
- Counting colors for *m* candidate *O(rm(l-c))*

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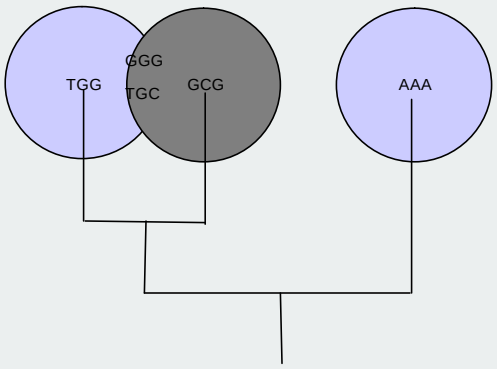
UPGMA + intersection



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